SUPPLEMENT FOR

Allosteric drug discrimination is coupled to mechanochemical changes in the Kinesin-5 motor core by E. Kim *et al.*

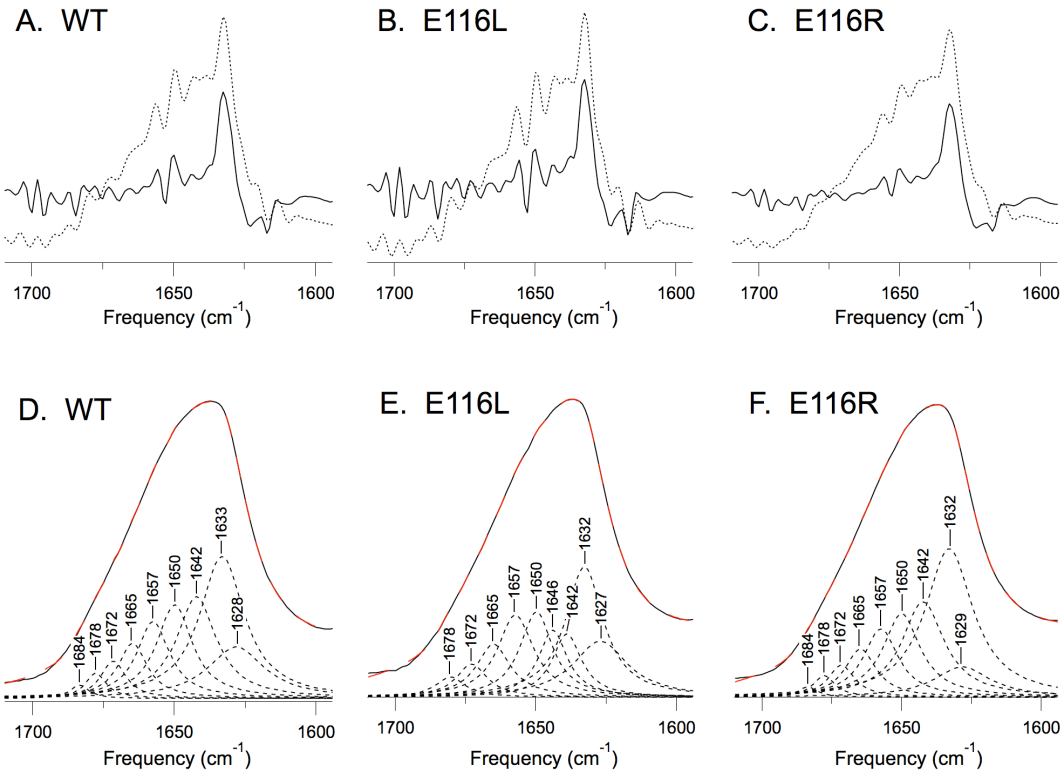

Fig. S1. **Band narrowing and regression analysis shows wildtype and E116R Eg5 spectra have nearly identical Lorentzians, while the E116L Eg5 spectrum loses a 1684 cm^{-1} component and acquires a 1644 cm^{-1} component.** Plotted are the 2nd derivative (solid), Fourier deconvolution (dashed) of (A) wildtype, (B) E116L, and (C) E116R amide I' bands. Peak fits were generated for (D) wildtype, (E) E116L, and (F) E116R spectra from these band narrowing calculations and the results are shown in dotted lines. The amide I' band of the averaged FTIR spectra (solid) is superimposed with the fitted trace (dashed red). Frequencies of the spectral components for all spectra were centered and binned to ±2 wavenumbers. Areas under the Lorenztian components were compiled and wildtype subtracted to result in spectrotype profiles of the structural data in Figure 3 and Supplementary Figure S2.

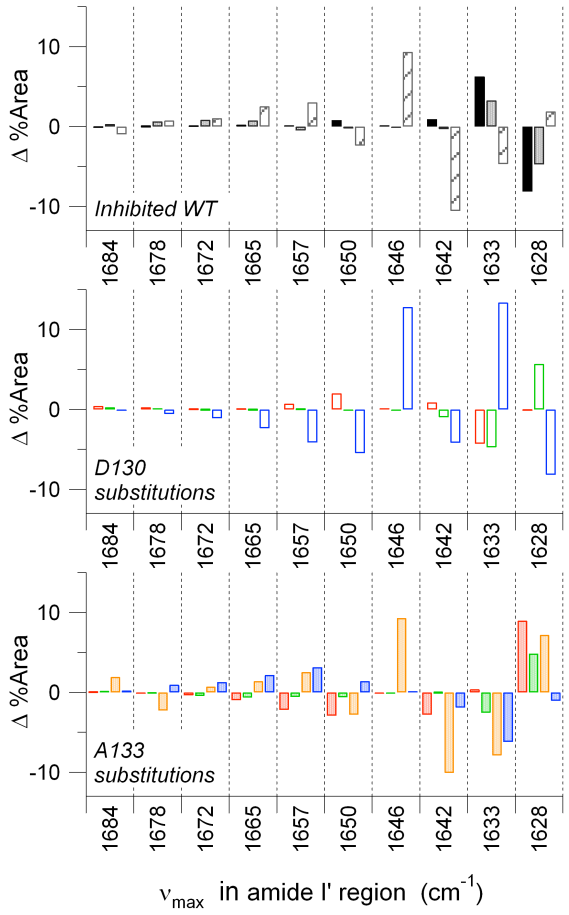

Fig. S2. **Spectrotypes of C-terminal L5 mutant proteins show larger variations in secondary structure changes in comparison to wildtype Eg5 kinesin.** Band narrowing of the amide I' spectral region, acquired from Eg5 proteins with residue substitutions in the C-terminus of the L5 loop, resulted in the same 10 frequency bins as the N-terminal L5 mutant proteins. Positive amplitudes indicate a gain of secondary structure from wildtype at a given frequency. Wildtype Eg5 kinesin was inhibited by both monastrol in 1:1 (black fill) and 1:20 (grey fill) ratios and STC (stripe fill). D130 substitutions include D130E (red), D130K (green), and D130V (blue). A133 substitutions are A133D (red), A133I (green), A133M (orange), and A133V (blue).

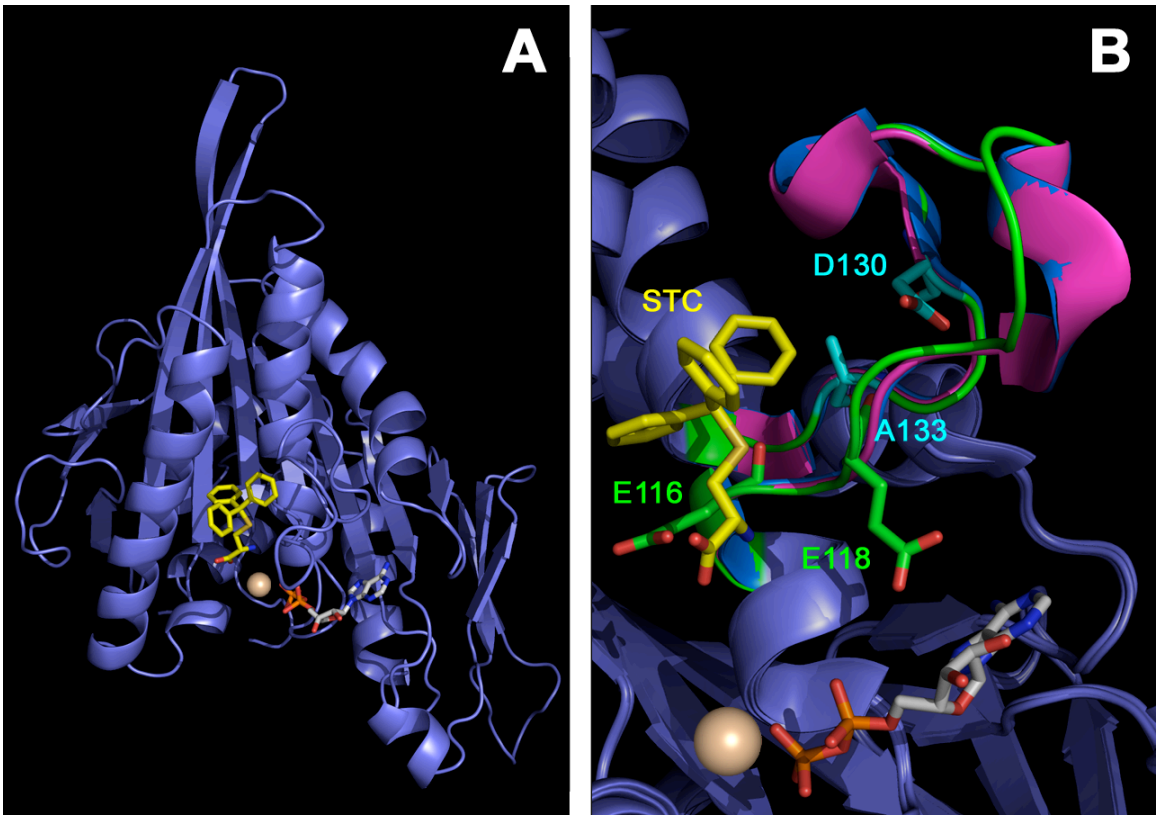

Fig. S3. **Comparison of X-ray crystallographic structures of the L5 loop of the HsEg5 motor domain bound with inhibitors show overall similarity.** Shown is (A) wildtype HsEg5 in complex with STC (PDB 3KEN). (B)The L5 loop exhibits a similar conformation with three different L5-directed inhibitors: STC in green, monastrol (PDB 1X88) in blue, and a tetrahydroisoquinoline carboxamide (PDB 2FME) in pink. Scored in green and aqua are E116/E118 and D130/A133 residues in the insertion loop, respectively. Also shown are STC (yellow), Mg^{2+} (gold sphere), and ADP. Monastrol contacts with Eg5 are largely mediated by nonpolar surfaces of the drug. Contact between motor domain residues and polar surfaces of the STC increases by over 20 $Å^2$, compared to monastrol ligation.

Movie S1. **Morph highlights changes in the antiparallel beta-sheet of Eg5•ADP from the STC-bound state to the monastrol-bound state.** Shown in the morph is a region of the central b-sheet of wildtype HsEg5 (grey) overlaid with the inhibitor-bound state (blue). At time zero, the blue structure is in the STC-bound state (PDB 3KEN). It proceeds through transformation into the monastrol-bound state (PDB 1X88), and back to the initial monastrol-bound state. This morph illustrates and highlights the structural changes in the beta strand distortions between the STC- and monastrol-bound states of Eg5.